SEEDLING RESPONSE TO ELEVATED CO$_2$ IN FIVE EPIGEAL SPECIES

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Evidence from numerous sources indicates that atmospheric CO$_2$ concentration has increased over the last 200 yr and will continue to increase in the foreseeable future. Much research is directed toward predicting the effects of this change on terrestrial vegetation. We quantified the effects of elevated CO$_2$ (700 μL L^{-1}) on the seedling growth characteristics of five C$_3$ species. These species all had epigeal germination morphology but differed by a factor of 20 in seed mass. The species and their respective seed-mass ranges were as follows: bagpod sesbania (*Sesbania vesicaria*), 0.23–0.26 g; cotton (*Gossypium hirsutum* var. Coker 317), 0.12–0.13 g; mesquite (*Prosopis glandulosa*), 0.041–0.048 g; cucumber (*Cucumis sativus* L. cv. Straight-8), 0.026–0.030 g; and hemp sesbania (*Sesbania exaltata*), 0.012–0.013 g. We measured increased total biomass in the elevated CO$_2$ environment for all species within 3 d of emergence. This rapid response contrasts with published reports that more time was required for a detectable response of seedlings to elevated CO$_2$. Growth analyses indicated that no single growth parameter predicted the responses of these species to elevated CO$_2$. However, maximum area of cotyledons was a better predictor of total biomass (at day 16) than was initial (day 0) seedling mass. Our data indicate that future increases in atmospheric CO$_2$ will greatly improve the competitive ability of C$_3$ epigeal species during the earliest growth stage in which cotyledons are still expanding.

Keywords: seedling morphology, epigeal, CO$_2$ response, seedling establishment.

Introduction

Predictions of future increases in atmospheric CO$_2$ concentration (Trabalka et al. 1986) have prompted numerous investigations into the potential effects of this change at levels ranging from the individual plant to the ecosystem. Even at the plant level, the effects of CO$_2$ enrichment are complex and interact with factors such as phenology and water and nutrient availability. Most studies to date have focused on plant response in later stages of development, in order to determine effects of CO$_2$ enrichment on reproductive output. Radoglou and Jarvis (1992, 1993) studied seedlings in the early stages of development and suggested that seedlings with small cotyledons (i.e., low mass seed) showed a response to elevated CO$_2$ earlier than seedlings with large cotyledons (large seed) because, in the latter, the large cotyledons provided substrate for growth for a longer period. The large-seeded species in their study was *Vicia faba* L., a hypogeal species, and the smaller-seeded species was *Phaseolus vulgaris*, an epigeal species. *Vicia faba* showed no positive response to elevated CO$_2$ during the first 45 d after emergence, while *Phaseolus vulgaris* showed positive effects of elevated CO$_2$ on biomass within 17 d of emergence.

Bunce (1997) studied growth parameters of C$_3$ crop and weed species and found that the ratio of total biomass at elevated versus ambient CO$_2$ concentrations was correlated with relative growth rate (RGR; dry mass increase [g g^{-1} d^{-2}]) at the ambient CO$_2$ concentration. Similarly, Poorter (1993) found that the stimulation in RGR was generally greater in species having higher RGR in a normal atmosphere. If this is a general phenomenon, then a relationship should exist between seed mass and magnitude of response to elevated CO$_2$ for the following reasons. First, Jurado and Westoby (1992), in a comparison of 32 Australian species, found that the RGR of seedlings from heavier-seeded species was lower than the RGR of seedlings from lighter-seeded species. They also cited extensive literature supporting this thesis (however, cf. Kelley 1996). Second, Seibert and Pearce (1993) compared growth and morphological characteristics of four weed and two crop species and found that the small-seeded weeds had higher RGRs than the crop species, presumably because they partitioned more seed reserves to the production of leaf area early in development. And finally, taken together, the reports of Radoglou and Jarvis (1992, 1993), Juardo and Westoby (1992), Seibert and Pearce (1993), and Bunce (1997) suggest that both the magnitude and the rapidity of response to elevated CO$_2$ may be influenced by seed mass.

Previous work has not considered the possible confounding influence of seedling morphology on response to CO$_2$ concentration. Lovell and Moore (1971) were among the first to discuss the influence of seedling morphology on early growth. In a study of four species with divergent developmental morphologies, they found that species with expanding cotyledons tended to accumulate biomass faster than species with non-expanding or nonphotosynthetic cotyledons. Species with non-expanding cotyledons produced true leaves at an earlier stage, but at the expense of rapid growth during the very early stages of development. Poorter and Remkes (1990) compared relative growth rates of 24 wild species and found a high correlation between leaf-area ratio (LAR; leaf area divided by total biomass) and RGR. This was despite the fact that the species they

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Table 1

Total Biomass (mg) of Four Species (Grown at 365 or 700 μL L^{-1} CO$_2$) 7–16 d after Emergence

Species and CO$_2$ concentration	Day 7	Day 10	Day 13	Day 16
Bagpod:				
365	268.2[A]	361.0[A]	724.5[A]	1248.0[A]
700	348.8[B]	515.8[B]	1179.4[B]	2077.5[B]
Cotton:				
365	154.1[A]	282.3[A]	557.6[A]	1150.4[A]
700	242.5[B]	436.6[B]	904.4[B]	1796.6[B]
Mesquite:				
365	45.3[A]	48.9[A]	79.7[A]	120.0[A]
700	48.7[A]	66.0[B]	112.5[B]	210.8[B]
Cucumber:				
365	169.2[A]	337.8[A]	757.29[A]	1672.9[A]
700	268.9[B]	662.5[B]	1279.3[B]	3048.2[B]

Note.   Values for a species and age not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).

studied included both monocots and dicots, annuals and perennials, and a broad array of seedling morphologies. Thus, seedling morphology and other factors need to be taken into consideration to clarify relationships between seed mass, seedling morphology, and response to CO$_2$ enrichment.

In addressing the question of the rate and magnitude of C$_3$ seedling response to elevated CO$_2$ as affected by seed mass, germination morphology (hypogeal or epigeal) should be controlled. Our objective was to test the prediction that the rapidity and magnitude of seedling response to elevated CO$_2$ is a function of seed mass; to this end, we used five epigeal species with a wide range in seed mass. Our second objective was to search for relationships between RGR and NAR (net assimilation rate; rate of dry-weight accumulation divided by leaf area) in five species grown at current and elevated CO$_2$.

Material and Methods

For each of the five species used in this study, large seed lots were assembled, and seeds were individually weighed so that a relatively narrow band of seed masses could be used for each species, thus reducing the effects generated by intraspecific variation in seed mass. This precaution was taken because we have observed significant correlations between seed mass and cotyledonary leaf area (cucumber) and seed mass and primary leaf area (bean; C. Tischler and H. Polley, unpublished data). The species and their respective seed-mass ranges were as follows: bagpod sesbania (*Sesbania vesicaria*), 0.23–0.26 g; cotton (*Gossypium hirsutum* var. Coker 317), 0.12–0.13 g; mesquite (*Prosopis glandulosa*), 0.041–0.048 g; cucumber (*Cucumis sativus* L. cv. Straight-8), 0.026–0.030 g; and hemp sesbania (*Sesbania exaltata*), 0.012–0.013 g. Cotton was obtained from the Texas Agricultural Experiment Station (Temple, Tex.), and cucumber was purchased locally. For the other three species, seed were hand collected from individual plants in central Texas in the fall of 1995.

Seeds of bagpod and hemp sesbania and mesquite were scarified before planting. Seeds were planted in plastic cones

(Steywe & Sons, Corvallis, Oreg.) that were 25 cm deep and 6.25 cm in diameter at the top. A commercial peat-perlite potting mix (Sun Grow Horticulture, Bellevue, Wash.) was employed. Total cone volume was ca. 425 cm^3. Cones were arranged in interlocking racks of 20. Planting dates were staggered to allow emergence to occur at about the same time for all species so that all species would experience similar light environments. Times from planting to emergence were as follows: mesquite and cucumber, 3 d; cotton and hemp sesbania, 4 d; and bagpod sesbania, 5 d. After planting, cones were watered weekly with full strength Hoagland's solution (Hoagland and Arnon 1950). At emergence, aberrant seedlings were discarded, and four healthy seedlings of each species were randomized within each rack. Aberrant seedlings were most common in cotton, where some degree of cotyledonary leaf damage from acid delinting was observed on every seedling. Seven plants per species were harvested at emergence (day 0) and on subsequent harvest dates. This "day 0" value, rather than mass of the dry seed, was used as the starting point for RGR analyses and as an estimate of seed mass. We thus avoided complications associated with the mass contribution of the inert seed coat, the mass of which tends to increase as total seed mass increases across species (Fenner 1983).

At each harvest date in each experiment, seedlings were cut at soil level, and shoots were separated into leaves and stems. Areas of cotyledonary and true leaves were determined with a photoelectric leaf-area meter. Potting mix was washed from the roots. Plant parts were dried 24 h at 65°C. Dry masses of roots, shoots, cotyledonary leaves, and true leaves were measured.

Two adjacent glasshouse bays at the Grassland, Soil, and Water Research Laboratory (Temple, Tex.) were utilized; CO$_2$ concentrations were either 365 or 700 μL L^{-1}. Air within each bay was sampled each 4 min with a Li-Cor (Lincoln, Nebr.) Model LI-622262 infrared gas analyzer to determine CO$_2$ concentration. Pure CO$_2$ was injected into the 700 μL L^{-1} glass-

Table 2

Total Biomass (mg) of Five Species 3 d after Emergence at 365 and 700 μL L^{-1} CO$_2$

Species and CO$_2$ concentration	Experiment 2	Experiment 3
Bagpod:		
365	159.6[A]	164.4[A]
700	167.9[A]	183.1[B]
Cotton:		
365	91.7[A]	109.5[A]
700	105.3[B]	132.8[B]
Cucumber:		
365	50.3[A]	68.9[A]
700	68.5[B]	91.4[B]
Mesquite:		
365	29.4[A]	33.8[A]
700	32.0[B]	38.7[B]
Hemp:		
365	11.2[A]	14.1[A]
700	13.4[B]	19.2[B]

Note.   If a pair of values in the same experiment column are not followed by the same letter, they are significantly different ($P = 0.05$).

Table 3

RGR and NAR for Five Species Calculated for 3–10 d after Emergence

Species and CO_2 concentration (μL L^{-1})	RGR (g g^{-1} d^{-1})	NAR (g m^{-2} d^{-1})
Bagpod:		
365	0.214	13.20
700	0.256	17.80
Cotton:		
365	0.421	19.45
700	0.442	26.20
Mesquite:		
365	0.131	9.31
700	0.182	13.38
Cucumber:		
365	0.282	9.24
700	0.331	13.48
Hemp:		
365	0.347	12.41
700	0.427	18.49

house bay as needed to maintain the target concentration. In the 365 μL L^{-1} bay, outside air was blown into the bay to maintain the desired ambient concentration. Air temperature was maintained near the outdoor temperature by periodic adjustment of thermostatic controls. The spring and summer of 1996 was unusually dry, and for all experiments described, plants experienced little overcast weather.

Experiment 1

Emergence occurred on April 1 and 2, 1996. Seedlings were sampled on days 7, 10, 13, and 16. Hemp sesbania was not included as an entry in this experiment.

Experiment 2

Data from experiment 1 indicated that, by the day 7 harvest, plants in the 700 μL L^{-1} atmosphere had significantly greater dry mass than those in the 365 μL L^{-1} atmosphere. Thus, days 3 and 5 after emergence were included as sampling times in experiment 2 in order to determine more precisely when responses to elevated CO_2 were observable. Results from experiment 1 indicated no differences in total biomass at emergence between the two CO_2 greenhouses, and thus day 0 cones (used to calculate initial seedling mass) were planted only in the 700 μL L^{-1} greenhouse. Planting dates were adjusted so that emergence occurred on May 5 and 6, 1996.

Experiment 3

Planting dates were adjusted such that emergence occurred on June 26 and 27, 1996. In this experiment, only day 3 data were collected for cotton, bagpod sesbania, cucumber, and mesquite. However, for hemp sesbania, adequate cones were planted to allow sampling at days 3, 5, 7, 10, and 16. These additional dates were included because hemp sesbania was not included in experiment 1, and additional data were needed to verify that the results of experiment 2 were indeed representative of growth of that species.

Data Analysis

Standard ANOVA and mean separation techniques were used to establish treatment differences. Relative growth rates were determined by standard methods. Initially, comparisons between species were made when species had a similar dry mass, as suggested by Coleman et al. (1994) and Poorter (1993). However, because of the large differences in seedling masses between species, this approach was abandoned. Average RGR and NAR values were calculated for the period of 3–10 d postemergence with data from experiment 2. This approach minimized possible problems created by developmental rests (Vogel 1980) or differences in growth rate related to developmental stage (Stebbins 1976).

Results

Experiment 1

By 7 d after emergence seedlings of bagpod sesbania, cotton, and cucumber in the 700 μL L^{-1} glasshouse bay were significantly higher in mass than analogous seedlings grown at the present CO_2 concentration (table 1). However, the difference was not significant in the case of mesquite. This may have been partially caused by inadequate sample number ($n = 5$) for this species at the 700 μL L^{-1} atmosphere and also by an aberrant seedling in this small sample. As expected, all differences between CO_2 atmospheres were significant for all species at all subsequent harvest dates (table 1). The species included in this experiment exhibited pronounced differences in biomass accumulation. The ratio of biomass at day 16 (data not shown) to that at day 0 (365 μL L^{-1} CO_2 atmosphere) was 16.9, 66.9, 8.5, and 4.4, for cotton, cucumber, bagpod sesbania, and mesquite, respectively.

Experiments 2 and 3

In experiment 2, total biomass at 3 d after emergence was significantly greater at 700 than at 365 μL L^{-1} CO_2 for all species except bagpod; and in experiment 3, it was greater in all cases (table 2). For subsequent harvests (days 5–16, ex-
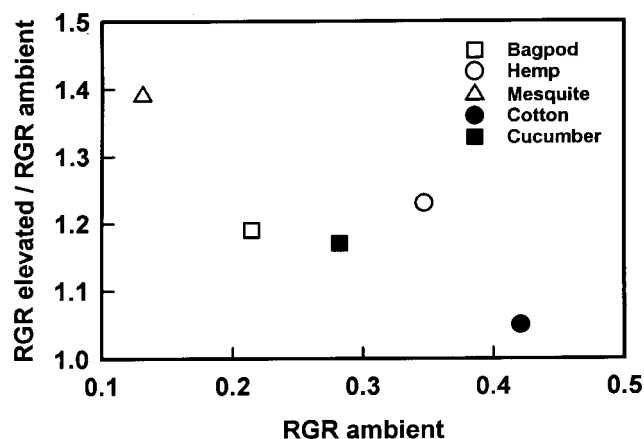

Fig. 1 Relationship between relative growth rate (RGR) at 365 μL L^{-1} CO_2 and the ratio of RGR at 700 μL L^{-1} CO_2 compared to that at 365 μL L^{-1} CO_2 for five species ($r^2 = 0.729$; $P = 0.0654$).

Table 4

Cotyledonary Leaf Area and Average Daily Increase in Biomass (First 16 d) of Five Species at Ambient and Elevated CO_2

		Biomass accumulation (mg)	
Species	Area (cm)	365 $\mu L\ L^{-1}\ CO_2$	700 $\mu L\ L^{-1}\ CO_2$
Bagpod ………	17.0	109	138
Cotton ……….	50.0	119	167
Cucumber ……	30.0	135	197
Mesquite …….	3.5	12	25
Hemp ………..	3.0	35	75

periment 2), the difference was significant in all cases. Biomass values were correspondingly higher in experiment 3 than in experiment 2 in every case, presumably because of the longer day lengths experienced in experiment 3. Behavior of hemp sesbania in experiment 3 (days 3–16) was very similar to that in experiment 2.

Data from experiment 2 was used to calculate average RGR and NAR values for days 3–10. For each species, RGR and NAR values were higher at elevated CO_2 (table 3). The relationship between RGR at current CO_2 concentration and relative stimulation in biomass at high versus current CO_2 (data not shown) was not significant (somewhat contrary to the findings of Bunce 1997). In fact, relative stimulation in biomass was fairly constant for all species except hemp sesbania, where stimulation was approximately twice that of the other four species. Also, there was no simple relationship between RGR at current CO_2 and absolute increase in RGR at elevated CO_2 (contrary to the pattern reported by Poorter 1993). There was a negative relationship between RGR at current CO_2 and the relative increase in RGR with increasing CO_2 ($P = 0.065$; fig. 1).

The relative increase in RGR was positively related to the relative stimulation in NAR at elevated CO_2 (table 3), which is consistent with Bunce (1997) but contrary to Poorter (1993). The relative increase in NAR, however, was not related to NAR at the current CO_2 level.

No relationship between initial seedling mass (day 0) and RGR (days 3–10) was apparent (data not shown). When mean LAR was determined from actual values calculated for all species and harvest dates, LAR was lower at elevated CO_2 in all but one case (3-d-old mesquite). Also, LAR was lowest at days 3 and 16 for all species except cucumber, where the highest LAR was observed at day 3 (data not shown), presumably because of the extent of cotyledonary leaf expansion in this species. Average daily biomass accumulation over the 16 d of experiment 2 for both CO_2 levels are presented along with maximum cotyledonary leaf area of the five species in table 4. Although rankings for maximum cotyledonary leaf area and biomass accumulation are not in strict rank order, a good correspondence between the two factors is apparent. In fact, Pearson correlation coefficients between maximum cotyledonary leaf area and biomass accumulation (for the entire 16 d) at 365 and 700 $\mu L\ L^{-1}\ CO_2$ were 0.812 ($P = 0.095$) and 0.809 ($P = 0.097$), respectively. These correlations were much better

than the corresponding correlations between initial seed mass (day of emergence) and biomass accumulation, 0.542 ($P = 0.3453$) and 0.401 ($P = 0.5031$), indicating that degree of cotyledon expansion is more important than mass at emergence in influencing biomass accumulation.

Discussion

Rapidity of Response to Elevated CO_2

The experiments described here demonstrate that for the five epigeal species studied significant responses to an atmosphere enriched to 700 $\mu L\ L^{-1}\ CO_2$ were observable within 3 d of emergence for four species and that responses were present in one out of two cases for a fifth species (bagpod). We saw little evidence for a relationship between seed mass and rapidity of response to elevated CO_2, at least in the range of seed masses (and within the single morphological type) we utilized. Our data do not directly conflict with those of Radoglou and Jarvis (1992, 1993), given that the large-seeded species they used exhibits hypogeal germination morphology and that it also has a seed mass substantially greater than that of the largest seed-mass species used in our experiments. Our ability to detect early responses to elevated CO_2 may be because we carefully controlled seed mass in our experiments. Because cotyledonary leaf area in cucumber is proportional to seed mass, differences in seed mass may generate variability that would not allow early detection of CO_2 responses. In addition, in the two cultivated species (cotton and cucumber) and three native species in our study, selection for seed mass was never performed, contrary to the situation in the species studied by Radoglou and Jarvis (1992, 1993). Consistent with the predictions of Lovell and Moore (1971), total area of cotyledonary leaves was a good predictor of rate of biomass accumulation (at least for the first 16 d of seedling growth).

Growth Analyses

The 20-fold difference in seed mass of the species we tested makes comparisons of RGR at a constant weight impossible because comparisons at the same seedling dry weight were complicated by large differences in phenology between the largest- and smallest-seeded species. Our approach of averaging from days 3 to 10 also tends to reduce effects of seedling "rests" (Vogel 1980) and transient differences in growth rate such as those observed by Stebbins (1976). Mesquite, the only woody species in this study, had the lowest RGR, but the value of 0.131 g $g^{-1}\ d^{-2}$ is substantially higher than mean RGR values presented by Hunt and Cornelissen (1997). In a study of 59 temperate plant species, they found a grand mean RGR of 0.09 g $g^{-1}\ d^{-2}$ for woody species. However, as our determinations were made early in development, when the mesquite seedling composition was more herbaceous than woody, one might expect an abnormally high RGR as compared to values determined later in development.

Contrary to Bunce (1997), our data showed no relationship between RGR at normal CO_2 and relative stimulation in biomass accumulation. Although the relationship between relative increase in RGR and relative stimulation of NAR at elevated CO_2 was not significant, this result was likely influenced by the species used in our study. Thus, we believe that interspecific

patterns in the response of biomass to CO_2 enrichment are likely to be more complicated than either Bunce (1997) or Poorter (1993) suggested and, in some cases, that these patterns may be species specific. Relative stimulation in biomass with CO_2 elevation is a function of both RGR at current CO_2 concentration and the increase in RGR at elevated CO_2. At the high light levels employed in this experiment, increase in RGR was correlated with relative increase in NAR, but increase in NAR was not easily predictable from NAR at present CO_2. Bunce (1997) found a good correlation between changes in NAR and response of leaf photosynthetic rate to CO_2, which suggests that a better understanding of mechanisms governing the photosynthetic response of seedlings to CO_2 will be necessary to predict seedling response to CO_2 enrichment.

Acknowledgments

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